Chromatin ImmunoPrecipitation

Grow cells: 50mL of ~0.5 OD_{600} per sample needed (~ 2.5-5 x10^8 cells)

Day 1:
Collect 50mL of OD_{600} 0.5 per sample

For each sample: @RT
Add 1.4 mL formaldehyde (1% final)
Rock for 15min
Add 3 mL 2.5M glycine (150mM final; neutralizes formaldehyde)
Rock for 5 min
(Place on ice if waiting for further time points)
Wash cells:
    Spin 5 min @ 3K @ 4°C
    Discard supernatant
    Resuspend in 25 mL **cold** TBS
Repeat wash
    **(the pellet can be snap frozen and kept at -80°C for later)
Resuspend in 400 µl FA-Lysis Buffer **(+ fresh protease inhibitors**)

(**keep samples on ice at all times**)
Prepare eppendorf tubes with 500 µl glass beads each **on ice**
Transfer cells to cold eppendorf tubes
Break cells with bead-beater for 40 min @ 4 ºC
Transfer to fresh eppendorf tubes (through a hole in the bottom of the first)
Resuspend any pellet by pipetting

Sonicate:
(Note: all sonicators are different. Times and velocities vary and should be tested for desired result of ~500 bp fragments. These setting are for a Fisher Scientific 550 Sonic Dismembrator)
Sonicate (on #4) for 10 sec ea., transfer to dry ice for 5 sec, then to wet ice ≥ 1 min
Repeat sonication a total of 5x per sample

Add 1mL FA-Lysis Buffer **(+fresh protease inhibitors**)
Spin for 30 min @ max @ 4 ºC
Transfer supernatant to new tubes
Spin 1 hr @ max @ 4 ºC
Transfer supernatant (the chromatin) to new tubes
    **(Snap freeze supernatant in aliquots and store at -80 ºC for later)

Split samples:
Inputs: 10 µl + 450 µl TE → store at -20 ºC for later
IPs: 100 µl + 300 µl FA-lysis (**+fresh protease inhibitor)** + Ab → O/N rocking @ 4 ºC (≥ 4 hrs) [vols. may vary to optimize pulldown]

(Equilibrate Beads overnight, see below)

Equilibrate Beads:
Wash 3x with 1ml TE (spin @10K for 30 sec)
Wash 3x with 1ml FA-lysis buffer
Resuspend to original volume with FA-lysis buffer

Day 2:
IPs only:
Add 6x volumes of Ab ($\geq$ 15 µl) of beads (protein-A sepharose if rabbit polyclonal Ab)
Incubate 1.5-2 hrs @ 4 °C on rocker
Wash: @RT
    Spin for 30 sec @ 6k xg
    Remove supernatant and discard (do **not** disturb bead-bed)
    Add 1.5 mL FA-lysis buffer
    Rock 5 min [time may vary to optimize]
Repeat washes sequentially with…
    2nd: FA-500 buffer
    3rd: LiCl wash buffer
    4th: TE
Resuspend in 250µl Elution buffer
Shake for 10 min @ 65 °C
Spin for 2 min @ max
Transfer supernatant to new tube
Add 250µl TE

IPs and Inputs:
reverse the crosslinking and treat with protease (either order)
    Incubate for at least 2 hrs @ 42 °C with 10µl proteinase K (0.2 ug/µl final)
    Incubate for at least 5 hrs @ 65 °C (usually O/N)

Day 3:
Purify DNA by 2 PCI extractions and 1 CI extraction:
    Add equal volume, vortex
    Spin 5 min @ max
    Extract aqueous layer (top) to a new tube
Transfer 100 µl of input DNA and 400µl of each sample to new tubes
Add 2.5 volumes EtOH, 1/10 volume 3M NaAcetate, pH5.2, and 1 µl glycogen (20mg/ml)
Mix samples by inversion
Incubate for 30 min @ -80 °C
Spin for 30 min @ max @ 4 °C
Discard supernatant
Dry pellet
Resuspend in water
    500 µl for Input DNA
    100 µl for IPs

qPCR

Buffers:

FA-Lysis Buffer	**[Final]**	**[Stock]**	**1L**
Hepes-KOH, pH7.5	50 mM	1 M	50 ml
NaCl	140 mM	5 M	28 ml
EDTA	1 mM	0.5 M	2 ml
Triton X-100	1%	10%	100 ml
Sodium Deoxycholate	0.1%	Powder	1.0 g
Protease Inhibitor cocktail (fresh)	1X	25X	
(Or Leupeptin & Pepstatin @ 1ug/ml & PMSF @ 1mM)			

FA-500 Buffer	[Final]	[Stock]	500 ml
Hepes-KOH, pH7.5	50 mM	1 M	25 ml
NaCl	500 mM	5 M	50 ml
EDTA	1 mM	0.5 M	1 ml
Triton X-100	1%	10%	50 ml
Sodium Deoxycholate	0.1%	Powder	0.5 g

LiCl Wash Buffer	[Final]	[Stock]	500 ml
Tris-HCl, pH 8.0	10 mM	1 M	5 ml
LiCl	250 mM	5 M	25 ml
NP-40	0.5%	10%	25 ml
Sodium Deoxycholate	0.5%	Powder	2.5 g
EDTA	1 mM	0.5 M	1 ml

2X Elution Buffer	[Final]	[Stock]	50 ml
Tris-HCl, pH 7.5	50 mM	1 M	2.5 ml
EDTA	10 mM	0.5 M	1 ml
SDS	1%	10%	5 ml

Adapted by
Papamichos-Chronakis, M., Petrakis, T., Ktistaki, E., Topalidou, I. & Tzamarias, D. Cti6, a PHD domain protein, bridges the Cyc8-Tup1 corepressor and the SAGA coactivator to overcome repression at GAL1. Mol. Cell 9, 1297–1305 (2002). | Article | PubMed | ISI | ChemPort |

from
M.H. Kuo and C.D. Allis, In vivo cross-linking and immunoprecipitation for studying dynamic Protein: DNA associations in a chromatin environment, *Methods* **19** (1999), pp. 425–433. Abstract | PDF (166 K) | View Record in Scopus | Cited By in Scopus (277)